26 ACETAMINOPHEN QUANTITATION AND CONFIRMATION BY LCMS

26.1 Summary

26.1.1 Acetaminophen is extracted from biological samples by making the samples weakly acidic/neutral with saturated sodium carbonate buffer and extracting with hexane/ethyl acetate. An aliquot of the extract is analyzed by high performance liquid chromatography-electrospray ionization mass spectrometry (LC-ESI-MS).

26.2 Specimen Requirements

26.2.1 0.5 mL blood, fluid or tissue homogenate.

26.3 Reagents and Standards

26.3.1 Acetaminophen, 1 mg/mL

26.3.2 Phenacetin, 1 mg/mL

26.3.3 Sodium carbonate ($NaHCO_3$)

26.3.4 Hexane

26.3.5 Ethyl acetate

26.3.6 Methanol

26.3.7 Acetic Acid

26.4 Solutions, Internal Standard, Calibrators and Controls

26.4.1 Saturated sodium carbonate solution. Add sodium carbonate to dH_2O until no more dissolves after shaking vigorously.

26.4.2 Hexane/Ethyl acetate (50:50, v:v). Mix 100 mL hexane with 100 mL ethyl acetate.

26.4.3 Drug stock solutions:

26.4.3.1 If 1 mg/mL commercially prepared stock solutions are not available, prepare 1 mg/mL solutions from powders. Weigh 10 mg of the free drug, transfer to a 10 mL volumetric flask and QS to volume with methanol. Note: If using the salt form, determine the amount of the salt needed to equal 10 mg of the free drug, and weigh this amount. Stock solutions are stored capped in a refrigerator and are stable for 2 years.

26.4.4 Working acetaminophen standard solution (0.2 mg/mL). Pipet 1 mL of 1 mg/mL acetaminophen stock solution into a 5 mL volumetric flask and QS to volume with methanol.

26.4.5 Working internal standard solution (50 µg/mL phenacetin): Pipet 500 µL of the 1 mg/mL stock solution of phenacetin into a 10 mL volumetric flask and QS to volume with methanol.

26.4.6 To prepare the calibration curve, pipet the following volumes of the 0.2 mg/mL acetaminophen stock solution into appropriately labeled 16 x 125 mm screw cap test tubes. Add 0.5 mL blank blood to obtain the final concentrations listed below.

Amount of 0.2 mg/mL acetaminophen (µL)	Final concentration of acetaminophen (mg/L)
250	100
150	60
100	40
50	20
25	10

26.4.7 Controls

26.4.7.1 Acetaminophen Control. Control may be from an external source or prepared in house using drugs from different manufacturers, lot numbers or prepared by a chemist different than the individual performing the extraction.

26.4.7.2 Negative control. Blood bank blood or equivalent determined not to contain acetaminophen or phenacetin.

26.5 Apparatus

26.5.1 Test tubes, 16 x 125 mm, round bottom, borosilicate glass with Teflon caps

26.5.2 Test tubes, 16 x 114 mm, glass centrifuge, conical bottom

26.5.3 Centrifuge capable of 2000-3000 rpm

26.5.4 Nitrogen evaporator with heating block

26.5.5 Vortex mixer

26.5.6 GC autosampler vials with inserts

26.5.7 LC/MS: Agilent Model 1100 LC-MSD

26.5.7.1 LCMS Instrument Conditions. The following instrument conditions may be modified to adjust or improve separation and sensitivity.

26.5.7.1.1 Elution Condicitons

26.5.7.1.1.1 Column: Agilent Hypersil BDS 125 mm X 3 mm, 3 µM particle size
26.5.7.1.1.2 Column thermostat: 30° C
26.5.7.1.1.3 Solvent A: 45% water with 1% acetic acid
26.5.7.1.1.4 Solvent B: 55% methanol
26.5.7.1.1.5 Isocratic elution, stop time: 4.0 min

26.5.7.1.2 Spray Chamber

26.5.7.1.2.1 Ionization Mode: Electrospray
26.5.7.1.2.2 Gas Temperature: 350° C

26.5.7.1.2.3 Drying Gas (N_2): 12.0 L/min
26.5.7.1.2.4 Nebulizer pressure: 30 psig
26.5.7.1.2.5 Vcap (Positive): 4000 V

26.5.7.1.3 Selected Ion Monitoring (quantitation ions)

26.5.7.1.3.1 Polarity: Positive
26.5.7.1.3.2 Injection volume: 2 µL

Time (min)	Group Name	SIM Ion	Frag-Mentor	Gain EMV	SIM Resol.	Actual Dwell
0.00	Acetaminophen	93	175	1.0	Low	218
		110	175		218	
		134	175		218	
		152	175		218	
2.30	Phenacetin	110	170	1.0	Low	292
		138	170		292	
		180	170		292	

26.6 Procedure

26.6.1 Label clean 16 x 125 mm screw cap tubes appropriately with calibrators, controls and case sample IDs.

26.6.2 Prepare calibrators and controls.

26.6.3 Add 0.5 mL case specimens to the appropriately labeled tubes.

26.6.4 Add 50 µL of the 50 µg/mL phenacetin internal standard working solution to each tube for a final concentration of 5 mg/L.

26.6.5 Add 1 mL saturated sodium carbonate buffer and 3 mL extraction solvent (50:50 hexane/ethyl acetate) to each tube.

26.6.6 Cap and rotate tubes for 30 minutes.

26.6.7 Centrifuge at approx 2500 rpm for 15 minutes. Transfer organic (upper) layer to appropriately labeled conical bottom test tubes.

26.6.8 Evaporate samples to dryness at approximately 60° C under nitrogen.

26.6.9 Reconstitute samples in 100 µL methanol. Transfer to GC autosampler vials for analysis by LCMS.

26.7 Calculation

26.7.1 Drug concentrations are calculated by linear regression analysis using the ChemStation software.

26.8 Quality Control and Reporting

26.8.1 See Toxicology Quality Guidelines

26.9 References

26.9.1 M Kennedy, D Sullivan and R Steiner, in house development